CHAPTER 13

Evolution of Resistance and Changes in Community Composition in Metal-Polluted Environments: A Case Study on Foundry Cove

Paul L. Klerks and Jeffery S. Levinton

TABLE OF CONTENTS

I. Introduction 224

II. Effects of Metals in Foundry Cove on the Community Composition 226

III. Resistance to Metals in Two Species of Macrobenthos of Foundry Cove 229

IV. Genetic Variation For Resistance in *Limnodrilus hoffmeisteri* from the Control Area as Determined by Heritability Estimates and a Selection Experiment 231

0-87371-734-1/93/$0.00 + $.50
© 1993 by Lewis Publishers

V. Discussion 233

VI. Future Directions 236
  A. Cost of Adaptation 236
  B. Number of Genes Explaining the Trait 237
  C. Molecular Studies of the Metallothionein Gene 237

Acknowledgments 238

References 238

I. INTRODUCTION

Environmental pollutants may have a variety of effects on communities. An alteration of the taxonomic composition is a commonly observed impact. For example, a reduction in the number of species has been reported for waters receiving metal-rich waste,[1-5] as well as for sites subject to both organic and inorganic pollutants.[6-8] Such a change in taxonomic composition of a community may be due to any of a wide variety of effects. These effects will be different for the multitude of species in a polluted site (due to differences in sensitivity among species or by virtue of the unique position that each species occupies in a foodweb), and may be as obvious as an increased mortality or reduced fecundity. For example, laboratory life-cycle exposures of crustaceans have shown reductions of the intrinsic rate of population growth by affecting survival, time till sexual maturity, number of broods, and brood development time.[9,10] But effects may be more subtle. For example, a toxicant may elicit an avoidance response by nonsessile organisms. This was demonstrated for the addition of hydrogen and aluminum to natural streams, which resulted in an increased emigration by several insect species.[11] A toxicant may also have an indirect effect on an organism. For example, an increase of predation in metal-dosed systems was shown for the predation of caddisflies by a stonefly,[12] and the predation of grass shrimp by killifish.[13] On the other hand, a reduction in growth of rainbow trout in insecticide-treated ponds was shown to be due to a reduction in prey abundance,[14] while an algal bloom in an insecticide-treated microcosm was due to the removal of grazers.[15]

The continued presence of a species in a polluted environment does not necessarily mean that pollutants are not having an effect (be it direct or indirect) on this species. For example, immigration of conspecifics from nearby unpolluted sites may be the basis of a population's survival in the polluted site. Another possibility is the occurrence of acclimation, where individuals become more resistant during their lifetime. Acclimation has been found for several populations and can be due, for instance, to the induction of detoxifying enzymes.[16,17] Finally, the continued presence of a species in a polluted environment despite an initial deleterious effect may be due to the evolution of resistance to the pollutant(s). This factor has obvious implications for long-term effects of both local pollution and global environmental changes, and will receive an in-depth treatment in this chapter.

The evolution of resistance is best known for resistance to pesticides in pest species. At least 504 species of insects or mites are known to be resistant to pesticides,[18] which demonstrates that resistance evolves commonly and rapidly. Factors that determine the occurrence and speed with which resistance to environmental pollutants evolves are expected to be similar to those identified for the development of resistance to pesticides.[19] The presence of heritable variation for resistance, selection pressures, immigration rates, etc. will all influence the evolution of resistance in a natural population that is exposed to an environmental pollutant. Resistance to environmental pollutants has been reported for several toxicants and species; for example, resistance to sulfur dioxide in *Geranium carolinianum*,[20] resistance to metals in several species of plants on abandoned mines,[21,22] resistance to metals in bacterial strains isolated from tanks used in the metal-processing industry,[23] and resistance to metals in an aquatic crustacean.[24] Yet insufficient data are available, especially for populations of animals, to determine how likely it is that resistance will evolve in polluted environments.[25,26] In addition, insight is lacking on the interplay between the evolution of resistance and changes at the community level brought about by an environmental pollutant. This study looked at both aspects of the effects of metals in invertebrates exposed to the very high levels of a few metals in Foundry Cove.

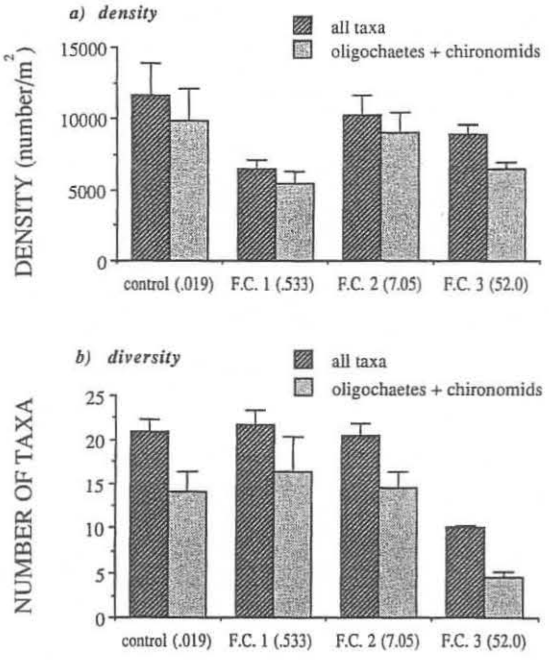
Foundry Cove is a tidal freshwater marsh on the Hudson River, located near Cold Spring (NY) approximately 87 km upriver from the mouth of the Hudson River. Waste from a nickel-cadmium battery factory was discharged into Foundry Cove from 1953 to 1971. Cobalt was released into the cove during some periods when it was being used as an additive. In spite of dredging in 1972 and 1973 to remove the most polluted sediment, cadmium levels as high as 50,000 μg Cd per g dry sediment were found in 1975.[27] Generally lower Cd concentrations were found in surface sediment in 1983, though surface concentrations approaching 40,000 μg/g Cd and subsurface Cd concentrations of 225,000 μg/g Cd were encountered.[28] Cadmium, nickel, and cobalt in Foundry Cove sediment generally occur in constant molar ratios of approximately 18:20:1, respectively.[28] The cadmium in Foundry Cove is bioavailable to plants, fish, and invertebrates.[29-31] Klerks[31] also reported accumulation of nickel by the benthic macrofauna, but did not find evidence that the cobalt was bioavailable. A nearby cove, South Cove, was used as a control site. Cadmium levels in this cove averaged about 19 μg/g, somewhat above baseline Cd levels but well below levels found in Foundry Cove.[28]

This study investigated effects of the metals in Foundry Cove on both the development of resistance and effects on the taxonomic composition of the benthic macrofauna. Effects on the composition of the macrobenthos were assessed by two taxonomic surveys. The development of resistance was determined by quantifying resistance in the oligochaete *Limnodrilus hoffmeisteri* and the chironomid *Tanypus neopunctipennis* collected from Foundry Cove, their offspring (for the oligochaete species), and animals collected from the control site. In addition, the presence of genetic variation for resistance was determined in *L. hoffmeisteri*, by quantifying the amount of heritable variation for metal resistance in the control population and by performing a laboratory selection experiment.

II. EFFECTS OF METALS IN FOUNDRY COVE ON THE COMMUNITY COMPOSITION

In an initial survey, benthic macrofauna samples were collected by taking sediment cores (surface area 35 cm^2) over a depth of 0 to 5 cm. Replicate samples were taken in the control area (19 μg/g Cd) and at three sites in Foundry Cove (approximately 500, 7000, and 52,000 μg/g Cd). At 4 of the sites, 5 cores were taken at 3 subsites that were approximately 10 m apart, while at the site with the highest Cd level (F.C.3, the original outfall site) only 1 set of 5 cores was taken as this site covered only a small area. The macrofauna was separated from the sediment using a 500 μm sieve, preserved, and later sorted under a dissecting microscope. The most abundant taxa, oligochaetes and chironomids, were usually identified to the species or genus level. Other taxonomic groups were generally identified at the family level. Results are reported here for the last two (October 11 and December 12, 1984) out of five sampling dates, since site F.C.3 was not included in the first three sampling dates. The existence of extremely high Cd levels (at F.C.3) was not discovered till the Fall of 1984. Analyses used subsite averages (means of five cores) and data for the two sampling dates were combined in the absence of significant differences among sampling dates and significant interactions between sampling date effects and site effects.

Densities of macrobenthic organisms did not differ significantly among the various sites (Figure 1a). This result held for the combination of all taxa, as well as the combination of oligochaetes and chironomids ($p > 0.05$ in ANOVA on log-transformed data). There is therefore no evidence that the high metal levels in Foundry Cove reduced the total number of organisms present. The situation was different however when the number of taxa were compared among the sites (Figure 1b). Both for all taxa combined as well as for oligochaetes and chironomids, the site with the highest Cd level showed a significantly reduced taxonomic diversity relative to all other sites ($p < 0.05$ in *a posteriori* pairwise comparisons on square root transformed data). The combination of an unchanged abundance and a reduced number of species means that some taxa are more

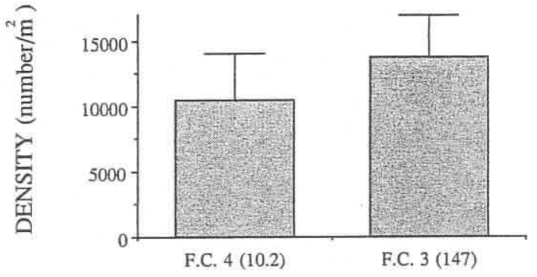


SITE (sediment Cd concentration in mg/g)

FIGURE 1. Densities of benthic macrofauna (a) and number of taxa (b) for all benthic organisms and the combination of oligochaetes + chironomids, in a site in the control area and three sites in Foundry Cove. Values are mean + S.E. for two sampling dates in 1984. Note that sediment Cd concentrations at the collection sites are reported in mg/g.

abundant at the outfall site (F.C.3). This was the case, for example, for the nematodes, the chironomid *T. neopunctipennis,* and the oligochaete *L. hoffmeisteri.* Absent from the outfall site were, for example, the chloromid tribe Tanytarsini and genera *Polypedilum, Einfeldia,* and *Cryptochironomus.* The chironomid genus *Procladius* also showed a statistically lower abundance at the outfall site, yet was present at this site. A possible complication of the conclusions on the taxonomic analyses could have been formed by differences in habitat type; the outfall site was located in a small cul-de-sac in Foundry Cove, while the other sites were located in the main parts of the cove. This left open the possibility that this difference in habitat-type was responsible for the reduced diversity in the outfall site rather than differences in metal levels.

To determine if the observed difference in diversity held up among sites with different metal levels yet similar in habitat-type, samples were collected on September 19, 1986 in both the outfall site and a nearby site also located in a cul-de-sac. In each site, 15 cores were taken. Sediment Cd concentrations in *a) density*



b) diversity

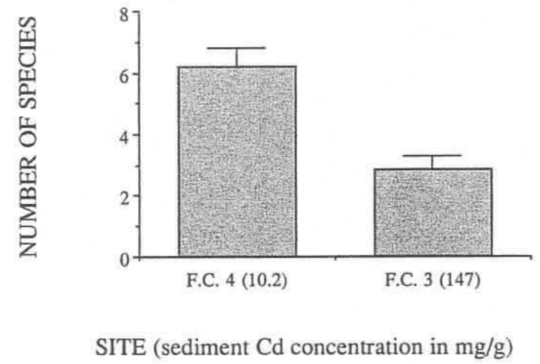


FIGURE 2. Densities of oligochaetes and chironomids combined (a) and number of chironomid and oligochaete species (b) in two sites in Foundry Cove with different metal levels. Values are mean + S.E. for samples collected in 1986. Sediment Cd concentrations were determined in the <500 μm fraction.

the <500 μm fraction of the sediment collected together with the macrobenthos, were 147,000 μg/g for the outfall site and 10,200 μg/g for the other site (F.C.4). Remember that the earlier survey had shown that the taxonomic diversity at a site with a Cd concentration of 7050 μg/g could not be distinguished from that at a control site. Results, based on averages per core, are similar to the results reported for the earlier survey. Densities of the combination of oligochaetes and chironomids (Figure 2a) did not differ among the sites ($p > 0.05$ in ANOVA on log-transformed data). Taxonomic diversities as expressed by the number of species (Figure 2b), were again lower at the site with the highest metal levels ($p < 0.001$ in ANOVA on $\sqrt{n}$-transformed data). It thus appears that the reduced diversity in the outfall site is indeed a consequence of the high metal levels at this site.

III. RESISTANCE TO METALS IN TWO SPECIES OF MACROBENTHOS OF FOUNDRY COVE

To determine if some of the macrobenthos species present in Foundry Cove exhibited an elevated resistance, laboratory bioassays were performed with two species: the chironomid *Tanypus neopunctipennis* and the oligochaete *Limnodrilus hoffmeisteri*. Animals collected in the control area and in the Foundry Cove site, with sediment Cd levels of about 7000 μg/g, were exposed in the laboratory to sediments from each site. *L. hoffmeisteri* was exposed for 28 days, using three replicates of 10 worms for each treatment. The chironomids were exposed for 14 days (to allow for their short life-cycle), starting the experiment with three replicates of 50 first-instar larvae (0 to 1 day old) per treatment. These larvae came from laboratory cultures that used sediment from the same site as the chironomids with which the cultures were started. All organisms were fed during the experiments. Survival was determined at the end of the exposure periods.

Results for *L. hoffmeisteri* (Figure 3a) show that these worms from Foundry Cove have an elevated resistance to the Cd-rich sediment; the difference in survival between worms from the two sites was highly significant ($p < 0.001$ in ANOVA on square root of number of survivors). None of the worms from the control area survived the exposure to Foundry Cove sediment, whereas 90% of the worms from Foundry Cove survived in this sediment. Experiments exposing worms from Foundry Cove and the control area to 8.9 μM Cd, 10.2 μM Ni, and 0.5 μM Co in water (the same ratio as observed for these metals in Foundry Cove sediment) confirmed that they are more resistant to the combination of Cd, Ni, and Co.[32]

The results were different for *T. neopunctipennis* (Figure 3b); no difference in survival could be detected ($p > 0.05$ in ANOVA on square root of number of survivors), though the Foundry Cove sediment resulted in a drastic reduction of the number of surviving chironomid larvae. Additional experiments with this species, exposing them for a longer part of their life-cycle (from larvae to adults or egg to adult stage), using more replicates per group and using sediment with Cd levels as high as 45,000 μg/g, did not result in the detection of differences in resistance between *T. neopunctipennis* from Foundry Cove and their conspecifics from the control area.[31]

As noted in the introduction, an increased resistance in organisms collected from a polluted site can be due to either acclimation or adaptation. To determine if the increased resistance to Cd-rich sediment in *L. hoffmeisteri* collected from Foundry Cove had a genetic basis, resistance in laboratory-reared offspring was investigated.[32] Worms from Foundry Cove were kept in clean sediment for two generations. Resistance to Cd-rich sediment was then compared among these offspring and worms collected from Foundry Cove and the control site. This was done in the same 28-day exposure procedure described earlier, except that sediments with a wider range in Cd concentrations were used in this experiment.

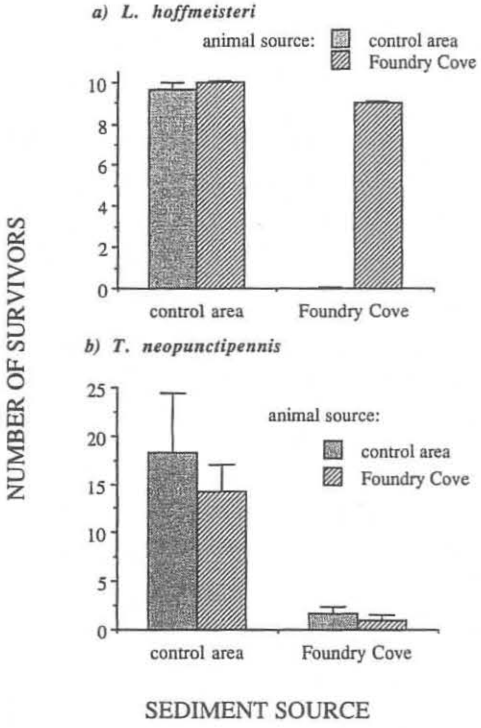


FIGURE 3. Resistance comparison for the oligochaete *L. hoffmeisteri* (a) and the larval stage of the chironomid *T. neopunctipennis* (b) between animals from the control site and individuals from Foundry Cove. Organisms from both sites were exposed to control sediment and metal-rich Foundry Cove sediment. Values are mean + S.E. (n = 3). Oligochaetes were exposed for 28 days (with 10 worms per replicate on day 0), while chironomids were exposed for 14 days (starting with 50 first-instar larvae). (Figure 3a is modified from Klerks, P. L. and Levinton, J. S., *Biol. Bull.*, 176, 135, 1989. With permission.)

Sediments with Cd concentrations ranging from about 20,000 to 90,000 μg/g were obtained by collecting sediment from various sites in Foundry Cove, sieving this to a less than 250 μm particle size, combining aliquots of sediment from different sites in various ratios, and stirring the combined sediment manually. Results of this experiment (Figure 4) show that very few of the worms survived the exposure to the sediment with Cd levels in excess of 50,000 μg/g. However, worms collected from Foundry Cove and their second generation offspring exhibited overall a significantly higher survival than the worms from the control area (in a multiple *t*-test at $\alpha = 0.05$ on square root of number of survivors). The results of this experiment thus show that the resistance in *L. hoffmeisteri* from Foundry Cove is largely genetically determined. Overall,

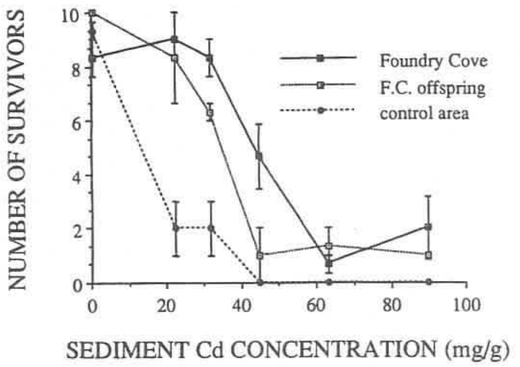


FIGURE 4. Resistance comparisons among *L. hoffmeisteri* from the control site, Foundry Cove, and second generation offspring of the Foundry Cove worms reared in the laboratory in control sediment. Worms were exposed for 28 days to sediment with various metal levels. Values are mean ± S.E. (n = 3, with 10 worms per replicate on day 0). (From Klerks, P. L. and Levinton, J. S., *Biol. Bull.*, 176, 135, 1989. With permission.)

no statistically significant difference in survival was detected between the worms collected from Foundry Cove and their offspring. However, the offspring tended to show a somewhat lower survival rate than the field-collected Foundry Cove worms. This reduction was statistically significant at one of the intermediate Cd levels, indicating a small environmental component in the resistance or a reduction in resistance upon relaxation of the selection pressure.

IV. GENETIC VARIATION FOR RESISTANCE IN *LIMNODRILUS HOFFMEISTERI* FROM THE CONTROL AREA AS DETERMINED BY HERITABILITY ESTIMATES AND A SELECTION EXPERIMENT

The genetic adaptation of *L. hoffmeisteri* to the high metal levels in Foundry Cove demonstrated that genetic variation for this resistance was initially present in this population. Frequency distributions of resistance among worms collected from specific sites in Foundry Cove (with resistance being determined as survival time upon exposure to a combination of Cd, Ni, and Co in reconstituted fresh water), indicated that the resistance in *L. hoffmeisteri* was a polygenic trait.[31] However, we do not have any more direct evidence on the number of genes involved in this resistance. Research addressing this issue is currently underway. For assessing the speed at which the evolution of resistance can occur, it is important to quantify the amount of genetic variation for resistance. This variation in a population not subjected to an intense selection was assessed by determining

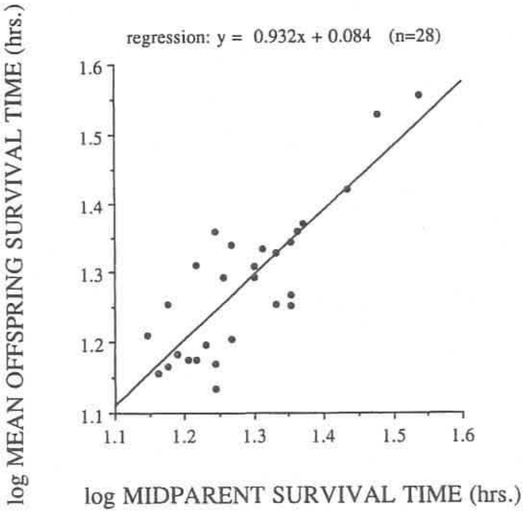


FIGURE 5. Relation between resistance in pairs of *L. hoffmeisteri* from the control site and their offspring. Resistance determined as survival time in water with 8.9 μ*M* Cd, 10.2 μ*M* Ni, and 0.5 μ*M* Co.

the heritability of resistance to the combination of Cd, Ni, and Co in the control population, using standard quantitative genetics procedures.[33] The heritability (also called narrow-sense heritability) of a trait is the proportion of the phenotypic variation that consists of additive genetic variation (i.e., the variation that selection acts upon).

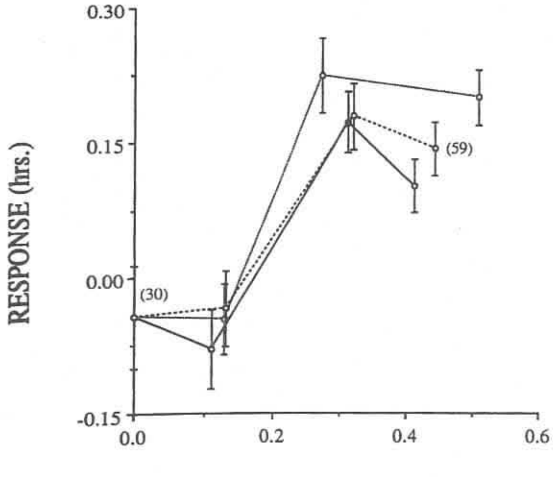
Animals were reared in pairs, and offspring were regularly separated from the parents. Metal resistance was then quantified by determining survival times of parents and offspring, in water spiked with 8.9 μ*M* Cd, 10.2 μ*M* Ni, and 0.5 μ*M* Co. The regression of mean offspring survival times on midparent survival times, shown in Figure 5, translates into a heritability of 0.93 ± 0.12 (S.E.). This is very close to the theoretical maximum of 1. Applying a weighting factor (based on the number of offspring in each family) to offspring mean resistance values resulted in a virtually unchanged heritability estimate of 0.92 ± 0.12. Another estimate, obtained from the regression of individual offspring survival times on individual parent values, was very high as well: 1.07 ± 0.10 (n = 455). Following the selection experiment (see below), a heritability estimate was obtained from the regression of the selection response on the cumulative selection differential (a cumulative measure of the selection applied). This realized heritability of 0.59 ± 0.14 was somewhat lower than the estimates obtained from the resemblances between relatives, but again indicated that a large part of the variation for resistance to the combination of Cd, Ni, and Co in the control population consisted of (additive) genetic variation. This means that the resistance in Foundry Cove could have evolved very rapidly, assuming similar heritabilities in this population prior to the metal waste discharge. For example, at a heritability of 0.6 and the top 40% in resistance of the population contributing to the next generation, the resistance observed in Foundry Cove would have evolved in about four generations.

The potential for the evolution of resistance in a control population was also addressed by performing a laboratory selection experiment. Three selection lines and one control line were started, with 441 juveniles each. Juveniles of the four lines came from the same source, pooled laboratory populations of worms from the control area. The three selection lines were exposed as adults to either metal-rich Foundry Cove sediment (in generation 0) or to Cd, Ni, and Co in water (in subsequent generations, this change being implemented to speed up the selection process). Surviving adults were allowed to reproduce in control sediment. Subsequently, the next generation was then started with 500 of their offspring, in each line. For the control line, the number of adults was reduced in each generation to the same (average) number that survived the selection exposures in the other lines. This was done to obtain the same effective population size (and thus inbreeding coefficient) in the control line as in the selected lines. The selection was continued for three generations and, on average, the individuals belonging to the top 14% in resistance were selected to contribute to the next generation. In each generation, resistance was quantified by determining survival times of adults exposed to 8.9 μM, 10.2 μM Ni, and 0.5 μM Co in reconstituted fresh water. The response to the selection was very rapid; a strong increase in resistance was obtained by the second generation (Figure 6).

The final difference in resistance between the selected lines and the control lines amounted to about 66% of the difference in resistance that was found between *L. hoffmeisteri* collected from Foundry Cove and their conspecifics from the control site. At this rate, the field-observed differences could have been obtained in four to five generations of selection at the conditions used in our laboratory selection procedure.

V. DISCUSSION

The demonstration of a reduced number of macrobenthic taxa at the most cadmium-, nickel-, and cobalt-polluted site in Foundry Cove is consistent with other studies in metal-polluted environments.[1-5] Surprisingly, no difference was found among the control area and the sites in the main part of Foundry Cove, despite sediment Cd levels as high as 7000 μg/g in the latter area. The fact that no difference in total number of taxa among these sites was detectable could be due to either a low metal-bioavailability, a low sensitivity of this parameter, or migration among sites. Other aspects of our study favor the explanation involving a low sensitivity of changes in the total number of taxa. It has been pointed out that such broad indices as species diversity are not very sensitive in detecting responses to stress, even though specific changes in species composition are among the earliest detectable responses.[34]



CUMULATIVE SELECTION DIFFERENTIAL

FIGURE 6. Response to selection in *L. hoffmeisteri* from the control site selected for an increased resistance to Cd, Ni, and Co during three generations. Response expressed as the difference in resistance (determined as survival time in 8.9 μ*M* Cd, 10.2 μ*M* Ni, and 0.5 μ*M* Co in water) between a selected line and the control line. Values are mean (with 95% confidence interval) of the difference with the control line, with the three different line symbols each referring to a different selection line; n = 60 for the determinations of survival times, except where noted otherwise. (From Klerks, P. L. and Levinton, J. S., *Biol. Bull.*, 176, 135, 1989. With permission.)

Our study did not detect any differences in total density of the macrobenthos among the differently polluted sites. This contrasts with studies conducted in Foundry Cove during 1973 and 1974.[35] The difference might reflect the reduction of Cd levels in Foundry Cove between 1974 and 1984, or could be related to the development of resistance in *L. hoffmeisteri*. If this species in Foundry Cove has become more resistant since 1974, a further increase in its abundance would have offset the lower abundance of other species. As mentioned earlier, our survey in 1984 showed that *L. hoffmeisteri* is much more abundant at the most polluted site than at the other sites.

The oligochaete *L. hoffmeisteri* in Foundry Cove was shown to have evolved a resistance to the high metal levels in this marsh. This adaptation has taken place in less than 30 years, demonstrating that response to selection in natural populations may be very rapid. This finding agrees with observations on the development of resistance in natural populations of pest species treated with pesticides. A review of the development of resistance to pesticides reported a rather narrow range of typically 5 to 50 generations of selection till resistance evolved, among numerous pesticide resistance cases.[36] Both the result of our selection experiment and the estimates of the heritability of resistance (of 0.59 to 1.07) demonstrate that resistance can evolve very rapidly. Similarly high heritabilities of resistance to stress have been reported by others: a heritability of 0.50 for resistance to sulfur dioxide in *Geranium carolinianum*,[37] and a heritability of 0.64 of resistance to desiccation in *Drosophila melanogaster*.[38]

The resistance in *L. hoffmeisteri* from Foundry Cove is not due to a reduced metal accumulation,[39] though this mechanism has been shown to be responsible for some cases of an increased metal resistance. For example, a copper-resistant strain of the alga *Ectocarpus siliculosus* was shown to accumulate less copper than their copper-sensitive conspecifics.[40] It appears that in worms from Foundry Cove, more Cd is detoxified by binding to a metallothionein-like protein than in *L. hoffmeisteri* from the control area.[39] In addition, Cd occurred in granules in worms from the metal-resistant population. Small (1-μm scale) granules and larger (up to 30 μm) granular aggregates were found in which Cd was possibly present as CdS. In addition, Cd was present in other small granules, possibly as a mixed calcium-cadmium-iron phosphate.[39]

Our resistance comparisons showed that *L. hoffmeisteri* in Foundry Cove has adapted to the high metal levels, whereas no resistance differences could be detected for the chironomid *T. neopunctipennis*. This difference between the two species could be due to the much lower metal accumulation in the chironomid than in the oligochaete.[31] Moreover, it is also expected that the amount of gene flow among sites will be higher for the chironomid (with a flying adult stage) than for the worm species. The choice of species to be included in the resistance comparisons was based on the availability of sufficient individuals for conducting experiments, as well as the success of maintaining laboratory cultures. For example, of the several species of chironomids brought into the laboratory, only *T. neopunctipennis* reproduced under the conditions that were used. The use of *L. hoffmeisteri* and *T. neopunctipennis* in the resistance comparisons meant that these comparisons were performed only with the two species that were most abundant at the site with the highest metal levels. Though the organisms used in the resistance comparisons were collected in a site in Foundry Cove where metal levels were lower (and where the two species in question did not exhibit an elevated abundance), it is unlikely that the resistance comparisons were representative of all the species present in the control area. Consequently, our study showed that some species may evolve a resistance to environmental pollutants, rather than demonstrating that resistance will evolve in many of the species in a polluted environment. Moreover, it has been pointed out that in order to assess the consequences of environmental change, one should worry about the species that do not evolve.[41] Now it has been shown by several investigations that some species can adapt to a changed environment, more insight might be obtained by investigating species that have disappeared from a changed environment.

VI. FUTURE DIRECTIONS

It has now been well established that evolutionary responses to toxic substances are common, but uneven in effects. Current research, however, has not been complete enough usually to understand the genetic architecture of adaptation to metals, nor have studies led to an understanding of potential interactions with neighboring populations. Our current understanding of the rapid evolution of resistance in *L. hoffmeisteri* permits exploration of some of these problems.

A. Cost of Adaptation

There clearly is much allelic variation for resistance in populations in relatively clean environments. This allows a rapid response to selection, as has been observed in Foundry Cove and in our selection experiments. We do not know, however, whether there is a **cost** to the adaptation to metals. We define cost as the reduction in fitness of a metal-adapted genotype. Owing to genotype-environment interactions we expect that the costs can be of two types: (I) costs associated with exposure to the metal; and (II) costs associated with the evolution of the adaptation itself. In the first case, the ability to sequester large amounts of cadmium may have extensive metabolic costs. Production of large amounts of the protein metallothionein may be costly, as may be the suite of transport processes used to sequester and eventually excrete the metal. Even when the animal is not exposed to metal, the possession of metal-resistance genes may have type II costs, since they may automatically induce a set of stereotyped physiological reactions which cost energy. It is possible, for example, that resistant strains automatically produce more sequestering organelles, which removes energy that might be allocated to other functions. Type II costs may also include changes in genes not related to metal resistance, but related to the selection process itself. For example, strong natural selection for metal resistance might involve the breakup of a strongly coadapted genome. In addition, genes linked to the genes involved in resistance might hitchhike to increased frequency in the population. This might be tested by an allozyme survey of the selected population, as compared to a metal-vulnerable population.

An understanding of these costs is important in assessing the potential for gene flow with other populations. If type I costs matter, resistant genotypes may do poorly in polluted habitats, and will contribute fewer offspring and hence fewer colonists to adjacent habitats. If type II costs are present, the resistant genotypes may disperse to other habitats, but they will not persist in very great abundance, since they are at an energetic disadvantage relative to local genotypes.

Type I costs would be encumbered only when the animal is exposed to metals. Type II costs may not be present, since the adaptation may possibly be explained by gene amplification. There is only limited evidence for amplification in insecticide resistance.[42] In *Drosophila melanogaster*, cases of gene duplication for the metallothionein gene are known, and these might influence adaptation to metals.[43] If gene multiples are selected in resistant populations, then the type II costs of adaptation may just involve the possession of more copies of a gene, which only involves synthesizing a little bit more DNA. However, type II costs may be substantial, even if resistance is due to gene amplification (for example, where the amplification results in elevated basal levels of metallothioneins).

B. Number of Genes Explaining the Trait

It is useful to understand the quantitative genetics of resistance, as we have demonstrated above for *L. hoffmeisteri*. The presence of resistant and susceptible populations also allows a study of the number of segregating genetic control elements, which we are now performing (Levinton and Martinez, in preparation). Such an analysis of resistance has been performed for the resistance to arsenic in the grass *Agrostis capillaris*.[44]

An algorithm to determine the number of segregating factors was first developed by Wright[45] and has been discussed succinctly with several applications.[46] Briefly, one crosses populations with different values of a trait to produce an F1 generation, and then calculates the increase of variance in the trait found in the F2. Lande[46] found that many morphological traits are underpinned by five to ten factors, which is a minimum estimate for the number of controlling genes. In cases where rapid evolution occurs, there is reason to believe that an allele at one locus might be recruited and would be the major source of evolutionary change. Major genes with modifiers, as observed for the As resistance in *A. capillaris*,[44] may be a common genetic architecture for traits under strong selection.

If only one factor underlies the resistance, then the trait is segregating as a Mendelian gene with two alleles. If we find this for *L. hoffmeisteri*, it is reasonable to conclude that the metallothionein gene is completely responsible for the genetic aspects of the evolution of resistance.

C. Molecular Studies of the Metallothionein Gene

Because a metallothionein-like protein is found in very high activity in the resistant strain of *L. hoffmeisteri*, we plan to investigate the molecular biology of adaptation by examining directly possible changes in the metallothionein gene(s), which have not yet been investigated in oligochaetes. Such genes have been identified and sequenced in several organisms, including the nematode *Caenorhabditis elegans*,[47] the fungus *Candida glabrata*,[48] the sea urchin *Strongylocentrotus purpuratus*,[49] and the fruit fly *Drosophila melanogaster*.[50] Using either protein purification and cloning or PCR, it should be possible to sequence the gene, and to search for cases of duplicates or sequence differences in the metallothionein genes of metal-adapted individuals.

ACKNOWLEDGMENTS

This research was financed, in part, through a research grant from the Hudson River Foundation for Science and Environmental Research, Inc. (New York, NY). This is contribution No. 816 from the Graduate Program in Ecology and Evolution of the State University of New York at Stony Brook.

REFERENCES

1. **Carpenter, K. E.,** A study of the fauna of rivers polluted by lead mining in the Aberystwyth district of Cardiganshire, *Ann. Appl. Biol.*, 11, 1, 1924.
2. **Winner, R. W., Boesel, M. W., and Farrell, M. P.,** Insect community structure as an index of heavy-metal pollution in lotic ecosystems, *Can. J. Fish. Aquat. Sci.*, 37, 647, 1980.
3. **Bryan, G. W. and Gibbs, P. E.,** Heavy metals in the Fal Estuary, Cornwall: A study of long-term contamination by mining waste and its effects on estuarine organisms, Mar. Biol. Assn. U.K., Plymouth, 1983.
4. **Malueg, K. W., Schuytema, G. S., Krawczyk, D. F., and Gakstatter, J. H.,** Laboratory sediment toxicity tests, sediment chemistry and distribution of benthic macroinvertebrates in sediments from the Keweenaw Waterway, Michigan, *Environ. Toxicol. Chem.*, 3, 233, 1984.
5. **Newell, R. C., Maughan, D. W., Trett, M. W., Newell, P. F., and Seiderer, L. J.,** Modification of benthic community structure in response to acid-iron wastes discharge, *Mar. Pollut. Bull.*, 22, 112, 1991.
6. **Butcher, R. W.,** Relation between the biology and the polluted condition of the Trent, *Int. Ver. Theor. Angew. Limnol. Verh.*, 12, 823, 1955.
7. **Reish, D. J.,** An ecological study of pollution in Los Angeles — Long Beach Harbors, California, *Occas. Pap. Allan Hancock Found.*, 22, 1, 1959.
8. **Becker, D. S., Bilyard, G. R., and Ginn, T. C.,** Comparisons between sediment bioassays and alterations of benthic macroinvertebrate assemblages at a marine superfund site: Commencement Bay, Washington, *Environ. Toxicol. Chem.*, 9, 669, 1990.
9. **Gentile, J. H., Gentile, S. M., Hoffman, G., Heltshe, J. F., and Hairston, N., Jr.,** The effects of a chronic mercury exposure on survival, reproduction and population dynamics of *Mysidopsis bahia*, *Environ. Toxicol. Chem.*, 2, 61, 1983.
10. **Van Leeuwen, C. J., Niebeck, G., and Rijkeboer, M.,** Effects of chemical stress on the population dynamics of *Daphnia magna:* A comparison of two test procedures, *Ecotoxicol. Environ. Saf.*, 14, 1, 1987.
11. **Hall, R. J., Driscoll, C. T., and Likens, G. E.,** Importance of hydrogen ions and aluminum in regulating the structure and function of stream ecosystems: An experimental test, *Freshwater Biol.*, 18, 17, 1987.
12. **Clements, W. H., Cherry, D. S., and Cairns, J., Jr.,** The influence of copper exposure on predator-prey interactions in aquatic insect communities, *Freshwater Biol.*, 21, 483, 1989.

13. **Kraus, M. L. and Kraus, D. B.,** Differences in the effects of mercury on predator avoidance in two populations of the Grass Shrimp *Palaemonetes pugio*, *Mar. Environ. Res.*, 18, 277, 1986.
14. **Crossland, N. O.,** A method for evaluating effects of toxic chemicals on the productivity of freshwater ecosystems, *Ecotoxicol. Environ. Saf.*, 16, 279, 1988.
15. **Taub, F. B.,** Demonstration of pollution effects in aquatic microcosms, *Int. J. Environ. Stud.*, 10, 23, 1976.
16. **Benson, W. H. and Birge, W. J.,** Heavy metal tolerance and metallothionein induction in fathead minnows: results from field and laboratory investigations, *Environ. Toxicol. Chem.*, 4, 209, 1985.
17. **Roesijadi, G., Drum, A. S., Thomas, J. M., and Fellingham, G. W.,** Enhanced mercury tolerance in marine mussels and relationship to low molecular weight, mercury-binding proteins, *Mar. Pollut. Bull.*, 13, 250, 1982.
18. **Georghiou, G. P.,** Overview of insecticide resistance, in *Managing Resistance to Agrochemicals*, Green, M. B., LeBaron, H. M., and Moberg, W. K., Eds., American Chemical Society, Washington, D.C., 1990, 18.
19. **Georghiou, G. P. and Taylor, C. E.,** Factors influencing the evolution of resistance, in *Pesticide Resistance: Strategies and Tactics for Management*, N.R.C. Committee on Strategies for the Management of Pesticide Resistant Pest Populations, Ed., National Academy Press, Washington, D.C., 1986, 157.
20. **Taylor, G. E., Jr. and Murdy, W. H.,** Population differentiation of an annual plant species, *Geranium carolinianum* L., in response to sulfur dioxide, *Bot. Gaz.*, 136, 212, 1975.
21. **Antonovics, J., Bradshaw, A. D., and Turner, R. G.,** Heavy metal tolerance in plants, *Adv. Ecol. Res.*, 7, 1, 1971.
22. **Verkleij, J. A. C. and Prast, J. E.,** Cadmium tolerance and co-tolerance in *Silene vulgaris* (Moench.) Garcke [= *S. cucubalus* (L.) Wib.], *New Phytol.*, 111, 637, 1989.
23. **Schmidt, T. and Schlegel, H. G.,** Nickel and cobalt resistance of various bacteria isolated from soil and highly polluted domestic and industrial wastes, *Microbiol. Ecol.*, 62, 315, 1989.
24. **Brown, B. E.,** Observations on the tolerance of the isopod *Asellus meridianus* Rac. to copper and lead, *Water Res.*, 10, 555, 1976.
25. **Klerks, P. L. and Weis, J. S.,** Genetic adaptation to heavy metals in aquatic organisms: a review, *Environ. Pollut.*, 45, 173, 1987.
26. **Klerks, P. L.,** Adaptation to metals in animals, in *Heavy Metal Tolerance in Plants: Evolutionary Aspects*, Shaw, A. J., Ed., CRC Press, Boca Raton, FL, 1989, 313.
27. **Kneip, T. J. and Hazen, R. E.,** Deposit and mobility of cadmium in a marsh-cove ecosystem and the relation to cadmium concentration in biota, *Environ. Health Perspect.*, 28, 67, 1979.
28. **Knutson, A. B., Klerks, P. L., and Levinton, J. S.,** The fate of metal contaminated sediments in Foundry Cove, New York, *Environ. Pollut.*, 45, 291, 1987.
29. **Hazen, R. E. and Kneip, T. J.,** Biogeochemical cycling of cadmium in a marsh ecosystem, in *Cadmium in the Environment. Part I: Ecological Cycling*, Nriagu, J. O., Ed., John Wiley & Sons, New York, 1980, 399.
30. **Hazen, R. E.,** *Cadmium in an Aquatic Ecosystem*, Ph.D. thesis, New York University, N.Y., 1981.

31. **Klerks, P. L. M.,** *Adaptation to Metals in Benthic Macrofauna,* Ph.D. thesis, State University of New York at Stony Brook, Stony Brook, 1987.
32. **Klerks, P. L. and Levinton, J. S.,** Rapid evolution of metal resistance in a benthic oligochaete inhabiting a metal-polluted site, *Biol. Bull.,* 176, 135, 1989.
33. **Falconer, D. S.,** *Introduction to Quantitative Genetics,* Longman, London, 1981.
34. **Schindler, D. W.,** Detecting ecosystem responses to anthropogenic stress, *Can. J. Fish. Aquat. Sci.,* 44 (Suppl. 1), 6, 1987.
35. **Occhiogrosso, T. J., Waller, W. T., and Lauer, G. J.,** Effects of heavy metals on benthic macroinvertebrate densities in Foundry Cove on the Hudson River, *Bull. Environ. Contam. Toxicol.,* 22, 230, 1979.
36. **May, R. M. and Dobson, A. P.,** Population dynamics and the rate of evolution of pesticide resistance, in *Pesticide Resistance: Strategies and Tactics for Management,* N.R.C. Committee on Strategies for the Management of Pesticide Resistant Pest Populations, Ed., National Academy Press, Washington, D.C., 1986, 170.
37. **Taylor, G. E., Jr.,** Genetic analysis of ecotypic differentiation within an annual plant species, *Geranium carolinianum* L., in response to sulfur dioxide, *Bot. Gaz.,* 139, 362, 1978.
38. **Parsons, P. A.,** Environmental stresses and conservation of natural populations, *Annu. Rev. Ecol. Syst.,* 20, 29, 1989.
39. **Klerks, P. L. and Bartholomew, P. R.,** Cadmium accumulation and detoxification in a Cd-resistant population of the oligochaete *Limnodrilus hoffmeisteri, Aquat. Toxicol.,* 19, 97, 1991.
40. **Hall, A., Fielding, A. H., and Butler, M.,** Mechanisms of copper tolerance in the marine fouling alga *Ectocarpus siliculosis.* Evidence for an exclusion mechanism, *Mar. Biol.,* 54, 195, 1979.
41. **Holt, R. D.,** The microevolutionary consequences of climatic change, *Trends Ecol. Evol.,* 5, 311, 1990.
42. **Devonshire, A. L. and Field, L. M.,** Gene amplification and insecticide resistance, *Annu. Rev. Entomol.,* 36, 1, 1991.
43. **Maroni, G., Wise, J., Young, J. E., and Otto, R.,** Metallothionein gene duplication and metal tolerance in natural populations of *Drosophila melanogaster, Genetics,* 117, 739, 1987.
44. **Watkins, A. J. and Macnair, M. R.,** Genetics of arsenic tolerance in *Agrostis capillaris* L., *Heredity,* 66, 47, 1991.
45. **Wright, S.,** *Evolution and the Genetics of Populations. Vol. 1, Genetic and Biometric Foundations,* University of Chicago Press, Chicago, 1968.
46. **Lande, R.,** The minimum number of genes contributing to quantitative variation between and within populations, *Genetics,* 99, 541, 1981.
47. **Imagawa, M., Onozawa, T., Okumura, K., Osada, S., Nishihara, T., and Kondo, M.,** Characterization of metallothionein cDNAs induced by cadmium in the nematode *Caenorhabditis elegans, Biochem. J.,* 268, 237, 1990.
48. **Mehra, R. K., Garey, J. R., Butt, T. R., Gray, W. R., and Winge, D. R.,** *Candida glabrata* metallothioneins, *J. Biol. Chem.,* 264, 19749, 1989.

49. **Harlow, P., Watkins, E., Thornton, R. D., and Nemer, M.,** Structure of an ectodermally expressed sea urchin metallothionein gene and characterization of its metal-responsive region, *Mol. Cell. Biol.*, 9, 5445, 1989.
50. **Mokdad, R., Debec, A., and Wegnez, M.,** Metallothionein genes in *Drosophila melanogaster* constitute a dual system, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 2658, 1987.